

## EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1	2-hydroxyethidium	US-PGPUB	OR	ON	2007/01/17 12:31
L2	1	2-hydroxyethid\$4	US-PGPUB	OR	ON	2007/01/17 12:31
L3	2	\$hydroxyethid\$4	US-PGPUB	OR	ON	2007/01/17 12:31
L4	53	hydroethid\$4	US-PGPUB	OR	ON	2007/01/17 12:32
L5	7806	superoxide	US-PGPUB	OR	ON	2007/01/17 12:33
L6	1	L2 WITH L5	US-PGPUB	OR	ON	2007/01/17 12:33
L7	10	L4 WITH L5	US-PGPUB	OR	ON	2007/01/17 12:34

## EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S2	2	2-hydroxyethidium	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/01/17 12:31
S4	6	hydroxyethid\$	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/01/11 15:00
S5	2	2-hydroxyethid\$	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2007/01/11 15:00

# STN search

\*\*\*\*\* STN Columbus \*\*\*\*\*

FILE 'HOME' ENTERED AT 08:21:38 ON 17 JAN 2007

=> file reg

COST IN U.S. DOLLARS

SINCE FILE

ENTRY

TOTAL

SESSION

FULL ESTIMATED COST

0.21

0.21

FILE 'REGISTRY' ENTERED AT 08:21:50 ON 17 JAN 2007

USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.

PLEASE SEE "HELP USAGETERMS" FOR DETAILS.

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provided by InfoChem.

STRUCTURE FILE UPDATES: 15 JAN 2007 HIGHEST RN 917470-98-5

DICTIONARY FILE UPDATES: 15 JAN 2007 HIGHEST RN 917470-98-5

New CAS Information Use Policies, enter HELP USAGETERMS for details.

TSCA INFORMATION NOW CURRENT THROUGH June 30, 2006

Please note that search-term pricing does apply when  
conducting SmartSELECT searches.

REGISTRY includes numerically searchable data for experimental and  
predicted properties as well as tags indicating availability of  
experimental property data in the original document. For information  
on property searching in REGISTRY, refer to:

<http://www.cas.org/ONLINE/UG/regprops.html>

=> ....Testing the current file.... screen

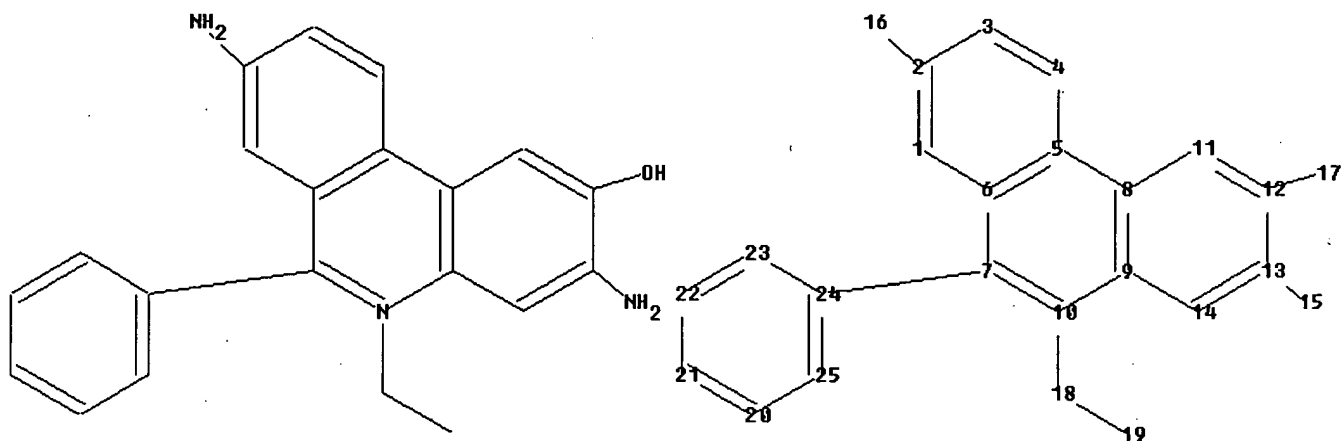
ENTER SCREEN EXPRESSION OR (END):end

=> screen 1569 AND 1700

L1 SCREEN CREATED

=>

Uploading C:\Program Files\Stnexp\Queries\hydroxyethidine.str



chain nodes :

15 16 17 18 19

ring nodes :

1 2 3 4 5 6 7 8 9 10 11 12 13 14 20 21 22 23 24 25

chain bonds :

2-16 7-24 10-18 12-17 13-15 18-19

ring bonds :

1-2 1-6 2-3 3-4 4-5 5-6 5-8 6-7 7-10 8-9 8-11 9-10 9-14 11-12 12-13  
13-14 20-21 20-25 21-22 22-23 23-24 24-25

exact/norm bonds :

2-16 10-18 12-17 13-15

exact bonds :

7-24 18-19

normalized bonds :

1-2 1-6 2-3 3-4 4-5 5-6 5-8 6-7 7-10 8-9 8-11 9-10 9-14 11-12 12-13  
13-14 20-21 20-25 21-22 22-23 23-24 24-25

isolated ring systems :

containing 1 : 20 :

Match level :

1:Atom 2:Atom 3:Atom 4:Atom 5:Atom 6:Atom 7:Atom 8:Atom 9:Atom 10:Atom  
11:Atom 12:Atom 13:Atom 14:Atom 15:CLASS 16:CLASS 17:CLASS 18:CLASS  
19:CLASS 20:Atom 21:Atom  
22:Atom 23:Atom 24:Atom 25:Atom

L2 STRUCTURE UPLOADED

=> que L2 AND L1

L3 QUE L2 AND L1

=> s 13

SAMPLE SEARCH INITIATED 08:22:08 FILE 'REGISTRY'

SAMPLE SCREEN SEARCH COMPLETED - 3 TO ITERATE

100.0% PROCESSED 3 ITERATIONS

SEARCH TIME: 00.00.01

0 ANSWERS

FULL FILE PROJECTIONS: ONLINE \*\*COMPLETE\*\*

BATCH \*\*COMPLETE\*\*  
PROJECTED ITERATIONS: 3 TO 163  
PROJECTED ANSWERS: 0 TO 0

L4 0 SEA SSS SAM L2 AND L1

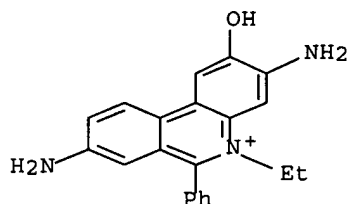
=> s l3 full  
FULL SEARCH INITIATED 08:22:32 FILE 'REGISTRY'  
FULL SCREEN SEARCH COMPLETED - 92 TO ITERATE

100.0% PROCESSED 92 ITERATIONS 1 ANSWERS  
SEARCH TIME: 00.00.01

L5 1 SEA SSS FUL L2 AND L1

=> d l5

L5 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2007 ACS on STN  
RN 854670-32-9 REGISTRY  
ED Entered STN: 12 Jul 2005  
CN Phenanthridinium, 3,8-diamino-5-ethyl-2-hydroxy-6-phenyl- (9CI) (CA INDEX  
NAME)  
MF C21 H20 N3 O  
SR CA  
LC STN Files: CA, CAPLUS, CASREACT, USPATFULL



\*\*PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT\*\*

8 REFERENCES IN FILE CA (1907 TO DATE)  
8 REFERENCES IN FILE CAPLUS (1907 TO DATE)

	SINCE FILE	TOTAL
	ENTRY	SESSION
=> file caplus		
COST IN U.S. DOLLARS		
FULL ESTIMATED COST	174.50	174.71

FILE 'CAPLUS' ENTERED AT 08:23:17 ON 17 JAN 2007

=> s l5  
L6 8 L5

=> d bib abs 1-8

L6 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2006:947192 CAPLUS Full-text

DN 145:501641

TI The confounding effects of light, sonication, and Mn(III)TBAP on quantitation of superoxide using hydroethidine

AU Zielonka, Jacek; Vasquez-Vivar, Jeannette; Kalyanaraman, B.

CS Department of Biophysics and Free Radical Research Center, Medical College of Wisconsin, Milwaukee, WI, 53226, USA

SO Free Radical Biology & Medicine (2006), 41(7), 1050-1057

CODEN: FRBMEH; ISSN: 0891-5849

PB Elsevier

DT Journal

LA English

AB Previously, the authors showed that hydroethidine (HE) reacts with intracellular superoxide radical anion ( $O_2^{\bullet-}$ ) to form a unique fluorescent marker product, 2-hydroxyethidium cation (2-OH-E<sup>+</sup>), that was not formed from HE reaction with other biol. relevant oxidants. Here they rigorously assessed the confounding effects of light, sonication, and Mn(III)TBAP on 2-OH-E<sup>+</sup>, the HE/ $O_2^{\bullet-}$  reaction product. Results indicate that continuous exposure to visible light induced photo-oxidation of HE to ethidium cation (E<sup>+</sup>) by a 2-OH-E<sup>+</sup>-dependent mechanism. Treatment of HE with ultrasound, a frequently used technique to lyse cell membranes, induced 2-OH-E<sup>+</sup> from in situ generation of  $O_2^{\bullet-}$ . Mn(III)TBAP, a cell-permeable metal-porphyrin complex used as a catalytic antioxidant, reacts with HE to form E<sup>+</sup>. This finding provides an alternative interpretation for Mn(III)TBAP effects during the HE/ $O_2^{\bullet-}$  reaction. In order to correctly interpret the HE reaction with  $O_2^{\bullet-}$  in cells, it is therefore imperative that HE and HE-derived products be measured by HPLC. A new and improved HPLC-electrochem. (HPLC-EC) detection has been developed for anal. of intracellular  $O_2^{\bullet-}$ . The HPLC-EC method is at least 10 times more sensitive than the HPLC-fluorescence technique for detecting  $O_2^{\bullet-}$  in cells.

RE.CNT 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2006:488099 CAPLUS Full-text

DN 145:310939

TI Endothelial cell superoxide anion radical generation is not dependent on endothelial nitric oxide synthase-serine 1179 phosphorylation and endothelial nitric oxide synthase dimer/monomer distribution

AU Whitsett, Jennifer; Martasek, Pavel; Zhao, Hongtao; Schauer, Dennis W.; Hatakeyama, Kazuyuki; Kalyanaraman, Balaraman; Vasquez-Vivar, Jeannette

CS Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI, 53226, USA

SO Free Radical Biology & Medicine (2006), 40(11), 2056-2068

CODEN: FRBMEH; ISSN: 0891-5849

PB Elsevier

DT Journal

LA English

AB Tetrahydrobiopterin (BH<sub>4</sub>) and heat shock protein 90 (hsp90) have been anticipated to regulate endothelial nitric oxide synthase (eNOS)-dependent superoxide anion radical ( $O_2^{\bullet-}$ ) generation in endothelial cells. It is not known, however, whether hsp90 and BH<sub>4</sub> increase  $O_2^{\bullet-}$  in a synergistic manner, or whether this increase is a consequence of downstream changes in eNOS phosphorylation on serine 1179 (eNOS-S1179) and changes in dimer/monomer distribution. Here  $O_2^{\bullet-}$  production from purified BH<sub>4</sub>-free eNOS and eNOS:hsp90 complexes determined by spin-trapping methodol. showed that hsp90 neither inhibits  $O_2^{\bullet-}$  nor alters the requirement of BH<sub>4</sub> to inhibit radical release from eNOS. In endothelial cells,  $O_2^{\bullet-}$  detection with the novel high-performance liquid chromatog. assay of 2-hydroxyethidium showed that

inhibition of hsp90 did not increase O2•-, while a significant increase in O2•- was detected in BH4-depleted cells. Radicicol, a hsp90 inhibitor, disrupted eNOS:hsp90 association, decreased eNOS-S1179, but increased biopterin production in a dose-dependent fashion. These changes were followed by an increase in eNOS activity, demonstrating that high biopterin levels offset inhibition of eNOS phosphorylation and diminished interaction with hsp90. In contrast, depletion of biopterin did not affect hsp90 levels or interaction with eNOS or eNOS dimer/monomer ratio in bovine aorta endothelial cells (BAECs). We conclude that low BH4 but not inhibition of hsp90 increases O2•- in BAECs by mechanism(s) that unlikely involve phosphorylation to eNOS-S1179 or eNOS monomerization.

RE.CNT 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2006:474498 CAPLUS Full-text

DN 145:308682

TI Definitive evidence for the nonmitochondrial production of superoxide anion by human spermatozoa

AU De Iuliis, Geoffrey N.; Wingate, Jordana K.; Koppers, Adam J.; McLaughlin, Eileen A.; Aitken, R. John

CS Centre of Excellence in Biotechnology and Development and Discipline of Biological Sciences, University of Newcastle, Callaghan, 2308, Australia

SO Journal of Clinical Endocrinology and Metabolism (2006), 91(5), 1968-1975  
CODEN: JCEMAZ; ISSN: 0021-972X

PB Endocrine Society

DT Journal

LA English

AB Oxidative stress in the male germ line was associated with poor fertility, impaired embryonic development, miscarriage, and childhood disease. Such stress is known to be associated with the peroxidn. of unsatd. fatty acids in the sperm plasma membrane and oxidative DNA damage to both the nuclear and mitochondrial genomes. However, the source of the free radicals responsible for such damage is still unresolved. The objective of this study was to chemical validate the use of dihydroethidium (DHE) as a probe for detecting the generation of superoxide anion by human spermatozoa and to examine the relationship between this activity and defective sperm function. DHE and SYTOX green were used in conjunction with flow cytometry and HPLC to investigate superoxide generation by human spermatozoa. Cause and effect relationships were established using menadione to artificially drive superoxide production by these cells. HPLC, mass spectrometry, NMR spectroscopy, and spectrofluorometry were used to demonstrate that human spermatozoa generate the superoxide-specific product, 2-hydroxyethidium, from DHE. Spontaneous superoxide production by human spermatozoa was found to originate from a nonmitochondrial source and was inversely correlated with sperm motility. A causative relationship between superoxide generation and sperm function was demonstrated when the pharmacol. stimulation of this activity with menadione was shown to result in both severe motility loss and DNA damage. These studies validate a methodol. for investigating the origins of oxidative stress in the male germ line and demonstrate, for the first time, the significance of superoxide generation by human spermatozoa in the etiol. of this condition.

RE.CNT 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2005:1233974 CAPLUS Full-text

DN 144:83346

TI An ultrasensitive fluorescent assay for the in vivo quantification of

superoxide radical in organisms

AU Georgiou, Christos D.; Papapostolou, Ioannis; Patsoukis, Nikolaos;  
Tsegenidis, Theodore; Sideris, Theodore

CS Department of Biology, Section of Genetics, Cell Biology, and Development,  
University of Patras, Patras, 26100, Greece

SO Analytical Biochemistry (2005), 347(1), 144-151  
CODEN: ANBCA2; ISSN: 0003-2697

PB Elsevier

DT Journal

LA English

AB Superoxide radical is a very important parameter of oxidative stress involved in a variety of biol. phenomena; therefore, its in vivo study is of utmost significance. However, its accurate detection is a challenge due to its short lifetime and its very low physiol. concentration. All current assays are qual. and nonspecific, and at best they are performed in vitro. The current dihydroethidine-based assay overcomes all these problems and introduces the following novelties. First, it measures the in vivo superoxide production in animals, plants, and microorganisms. Second, it is ultrasensitive and very simple in that it can measure superoxide radical as low as 1.5 pmol in biol. samples as low as 5 mg. Third, the very high sensitivity of the assay renders possible, for the first time, the measurement of the actual rate of formation of superoxide radical under physiol. and simulated nonphysiol. conditions.

RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2005:1103255 CAPLUS Full-text

DN 143:362879

TI Methods for the preparation and isolation of 2-hydroxyethidium and use in hydroethidine-based superoxide detection as standard

IN Kalyanaraman, Balaraman; Zhao, Hongtao

PA USA

SO U.S. Pat. Appl. Publ., 20 pp.  
CODEN: USXXCO

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2005227307	A1	20051013	US 2004-820599	20040408
PRAI	US 2004-820599		20040408		

AB The inventors have purified the fluorescent product in the hydroethidine-based superoxide detection assays and further identified the product as 2-hydroxyethidium. Methods for synthesizing 2-hydroxyethidium and for detecting and quantifying superoxide are provided.

L6 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2005:978284 CAPLUS Full-text

DN 144:107882

TI Mechanistic similarities between oxidation of hydroethidine by Fremy's salt and superoxide: stopped-flow optical and EPR studies

AU Zielonka, Jacek; Zhao, Hongtao; Xu, Yingkai; Kalyanaraman, B.

CS Department of Biophysics and Free Radical Research Center, Medical College of Wisconsin, Milwaukee, WI, 53226, USA

SO Free Radical Biology & Medicine (2005), 39(7), 853-863  
CODEN: FRBMEH; ISSN: 0891-5849

PB Elsevier

DT Journal

LA English



OS CASREACT 144:107882

AB The authors have previously shown that superoxide radical anion ( $O_2^{\bullet-}$ ) reacts with hydroethidine (HE) to form a product that is distinctly different from ethidium ( $E^+$ ) (Zhao et al., Free Radic. Biol. Med. 34:1359; 2003). The structure of this product was recently determined as the 2-hydroxyethidium cation ( $2-OH-E^+$ ) (Zhao et al., Proc. Natl. Acad. Sci. USA 102:5727; 2005). Using HPLC and mass spectrometry techniques,  $2-OH-E^+$  is formed from the reaction between HE and nitrosodisulfonate radical dianion (NDS) or Fremy's salt. The reaction kinetics and mechanism were determined using steady-state and time-resolved optical and EPR techniques. Within the first 50 ms, an intermediate was detected. Another intermediate absorbing strongly at 460 nm and weakly at 670 nm was detected within a second. The structure of this species was assigned to an imino quinone derivative of HE. The stoichiometry of the reaction indicates that two mols. of NDS were needed to oxidize a mol. of HE. The authors postulate that the first step of the reaction involves the hydrogen atom abstraction from HE to form an aminyl radical that reacts with another mol. of NDS to form an adduct that decomps. to an imino quinone derivative of HE. A similar mechanism was proposed for the reaction between HE and  $O_2^{\bullet-}$ . The reaction between HE and the Fremy's salt should provide a facile route for the synthesis of  $2-OH-E^+$ , a diagnostic marker product of the  $HE/O_2^{\bullet-}$  reaction.

RE.CNT 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2005:600020 CAPLUS Full-text

DN 143:434254

TI Detection and characterization of the product of hydroethidine and intracellular superoxide by HPLC and limitations of fluorescence. [Erratum to document cited in CA143:055106]

AU Zhao, Hongtao; Joseph, Joy; Fales, Henry M.; Sokoloski, Edward A.; Levine, Rodney L.; Vasquez-Vivar, Jeannette; Kalyanaraman, B.

CS Department of Biophysics and Free Radical Research Center, Medical College of Wisconsin, Milwaukee, WI, 53226, USA

SO Proceedings of the National Academy of Sciences of the United States of America (2005), 102(25), 9086

CODEN: PNASA6; ISSN: 0027-8424

PB National Academy of Sciences

DT Journal

LA English

AB The last sentence of the abstract, "We conclude that the HPLC/fluorescence assay using HE as a probe is more suitable reactive oxygen species for detecting intracellular  $O_2^{\bullet-}$ ," should read: "We conclude that the HPLC/fluorescence assay using HE as a probe is more suitable for detecting intracellular  $O_2^{\bullet-}$ ." This change does not alter the conclusions of the article.

L6 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2005:372758 CAPLUS Full-text

DN 143:55106

TI Detection and characterization of the product of hydroethidine and intracellular superoxide by HPLC and limitations of fluorescence

AU Zhao, Hongtao; Joseph, Joy; Fales, Henry M.; Sokoloski, Edward A.; Levine, Rodney L.; Vasquez-Vivar, Jeannette; Kalyanaraman, B.

CS Department of Biophysics and Free Radical Research Center, Medical College of Wisconsin, Milwaukee, WI, 53226, USA

SO Proceedings of the National Academy of Sciences of the United States of America (2005), 102(16), 5727-5732

CODEN: PNASA6; ISSN: 0027-8424

PB National Academy of Sciences

DT Journal

LA English

AB Here we report the structural characterization of the product formed from the reaction between hydroethidine (HE) and superoxide ( $O\bullet-2$ ). By using mass spectral and NMR techniques, the chemical structure of this product was determined as 2-hydroxyethidium (2-OH-E<sup>+</sup>). By using an authentic standard, we developed an HPLC approach to detect and quantitate the reaction product of HE and  $O\bullet-2$  formed in bovine aortic endothelial cells after treatment with menadione or antimycin A to induce intracellular reactive oxygen species. Concomitantly, we used a spin trap, 5-tert-butoxycarbonyl-5-methyl-1-pyrroline N-oxide (BMPO), to detect and identify the structure of reactive oxygen species formed. BMPO trapped the  $O\bullet-2$  that formed extracellularly and was detected as the BMPO-OH adduct during use of the EPR technique. BMPO, being cell-permeable, inhibited the intracellular formation of 2-OH-E<sup>+</sup>. However, the intracellular BMPO spin adduct was not detected. The definitive characterization of the reaction product of  $O\bullet-2$  with HE described here forms the basis of an unambiguous assay for intracellular detection and quantitation of  $O\bullet-2$ . Anal. of the fluorescence characteristics of ethidium (E<sup>+</sup>) and 2-OH-E<sup>+</sup> strongly suggests that the currently available fluorescence methodol. is not suitable for quantitating intracellular  $O\bullet-2$ . We conclude that the HPLC/fluorescence assay using HE as a probe is more suitable reactive oxygen species for detecting intracellular  $O\bullet-2$ .

RE.CNT 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> log y

COST IN U.S. DOLLARS

SINCE FILE	TOTAL
ENTRY	SESSION
23.11	197.82

FULL ESTIMATED COST

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)

SINCE FILE	TOTAL
ENTRY	SESSION
-6.24	-6.24

CA SUBSCRIBER PRICE

STN INTERNATIONAL LOGOFF AT 08:23:48 ON 17 JAN 2007